

# BORON-DOPED DIAMOND ELECTRODES FOR NITRIC OXIDE DETECTION

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## OBJECTIVE

We intend to characterize boron-doped diamond (BDD) microelectrodes for nitric oxide (NO) detection using flow injection analysis. Our ultimate goal is to utilize our electrodes for localized, *in vitro*, real-time NO detection.

## BACKGROUND & RATIONALE

Nitric oxide is a free radical signal molecule involved in many physiological functions and pathological changes in the nervous system, immune system, epithelial systems, smooth muscles and other tissues. In the retina, NO is a neurotransmitter synthesized by enzymes; it is also associated with a number of detrimental ocular conditions including uveitis and glaucoma<sup>1</sup>. Ability to measure NO reliably in real-time will advance the understanding of physiological and pathological processes in the retina. Typical NO detection methods, see **Ref 2**, include chemiluminescence and colorimetry, although these provide indirect NO measurement and cannot be used *in vitro* for localized, real-time sensing. Electrochemical (*e.g.*, cyclic voltammetry, amperometry) detection of NO is appealing because it may directly measure NO concentration and is inexpensive, sensitive, and relatively non-destructive to biological surroundings. The lower detection limit at typical electrodes (*e.g.*, carbon-fiber,<sup>3,4</sup> glassy carbon, pyrolytic graphite, and gold) for prepared NO solutions is  $\sim 1\ \mu\text{M}$ ; this limit varies with electrode material, surface modification scheme, and whether oxidation or reduction of NO is implemented for detection. NO's fleeting existence (seconds) and overall reactive environment in the retina make *in vitro*, real-time, selective, electrochemical detection difficult using aforementioned electrode materials.

Another major issue is that nitrite,  $\text{NO}_2^-$ , the primary interfering species for NO detection, undergoes its oxidation at a similar potential ( $< 100\text{mV}$  potential difference); thus, convolution of the response from a NO and  $\text{NO}_2^-$  mixture is necessary. Electrode surface modifications are typically used to tackle this problem; modifications,<sup>5,6</sup> *e.g.*, by metalloporphyrin or metalloprotein layers, to catalyze electron transfer and enhance sensor selectivity may significantly limit sensor response time. Other possible interferers in the retina include dopamine and acetylcholine.

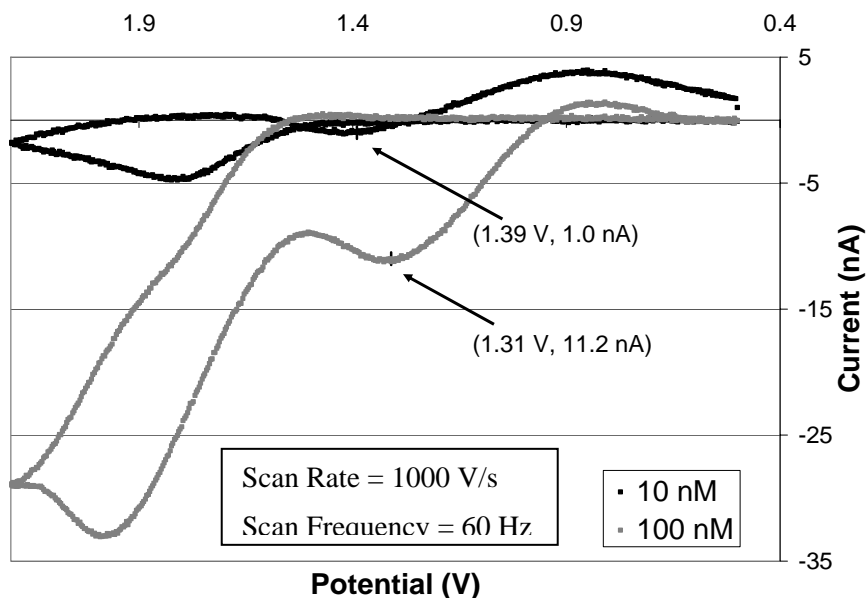
Diamond's extreme chemical stability should provide advantage for long-term, electrochemical detection in biological media.<sup>7</sup> In addition, diamond has been shown<sup>8</sup> to slow oxygen reduction, another interfering reaction in biochemical detection. Electrochemical detection of NO on a bare, BDD macroelectrode (area,  $0.07\ \text{cm}^2$ ) using slow scan cyclic voltammetry ( $20\ \text{mV/s}$ ) has been reported by Spataru *et al.*<sup>9</sup> The detection was not selective for NO over  $\text{NO}_2^-$ ; the  $\text{NO}_2^-$  detection limit was  $< 50\ \text{nM}$ . No reports of NO detection on BDD microelectrodes are known. Using our CVD techniques, we can fabricate sufficiently small microelectrodes to enable localized, *in vitro* detection of NO.

## METHODS & RESULTS

Boron-doped diamond macro- and microelectrodes were fabricated via hot-filament CVD. BDD macroelectrodes detected NO and  $\text{NO}_2^-$  oxidation, by slow scan voltammetry (scan rate,  $\sim 200\ \text{mV/s}$ ). Diamond microelectrodes were characterized for NO and  $\text{NO}_2^-$  detection in a flow cell system using fast-scan cyclic voltammetry (FSCV, scan rates, *ca.*  $100\ \text{V/s}$ ) to simulate neurotransmission (ms) time scales; the effect of scan rate on current responses was studied. We believe this is the first report of FSCV for real-time NO detection; typically amperometry<sup>3</sup> and differential normal pulse voltammetry (DNPV)<sup>4</sup> have been used. Carbon-fiber microelectrodes were controls for these experiments. Diamond surface modification schemes from diazonium salt precursors (see **Ref 10**), including enzyme attachment, were used to best maintain ultrasensitivity and promote selectivity. NO detection in retinal tissue with BDD microelectrodes was also pursued.

Initial experiments using BDD macroelectrodes verified (data not shown) the ability to detect NO (nominally  $10\ \mu\text{M}$ ) in prepared NO-containing electrolytes; BDD also appeared to display good selectivity of NO to  $\text{NO}_2^-$ . Selectivity was only observed on macroelectrodes; the source of this behavior is likely linked to surface chemistry. For FSCV flow injection, carbon-fiber microelectrodes had a NO detection limit of  $\sim 1\ \mu\text{M}$ , consistent with literature

for amperometry<sup>3</sup> and DNPV<sup>4</sup>. BDD microelectrodes were more sensitive, yielding a NO detection limit of ~10 nM (See **Fig 1**); unfortunately, the sensitivity decreased over time. NO peak current was found to vary with the square root of the scan rate, in agreement with Spataru *et al.*,<sup>9</sup> for NO as a non-adsorbing species. Our data suggest that the initial sensitivity and stability of BDD electrodes are affected by growth conditions and surface chemistry. Progress with our ongoing surface chemistry strategies as a means to understand these relationships will be presented.



**Figure 1.** Flow injection analysis of Nitric Oxide on a Boron-Doped Diamond Microelectrode – (NO dissolved in 0.1 M Phosphate Buffer Solution). NO peaks in +1.3 – +1.4 V range; Peak current ratio  $\approx$  NO concentration ratio.

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